

AMENDMENTS TO THE CLAIMS

1. (Currently Amended) A An isolated peptide selected from the following (a), (b), or (c) or (d):

- (a) a peptide consisting of the amino acid sequence as shown in SEQ ID NO: 4;
- (b) ~~a peptide which consists of the amino acid sequence as shown in SEQ ID NO: 4 having addition, deletion or substitution of one or a plurality of amino acids and has β-ionone ring-2-hydroxylase activity;~~
- (e) (b) a peptide which is isolated from a naturally occurring bacterium and which consists of an amino acid sequence having a 50% or more identity with the amino acid sequence as shown in SEQ ID NO: 4 and has β-ionone ring-2-hydroxylase activity; or
- (d) (c) a bacterium-derived peptide which is encoded by a DNA consisting of the nucleotide sequence as shown in SEQ ID NO: 3 or a DNA that hybridizes to the full complement of SEQ ID NO:3 hybridizable to a complementary DNA to said DNA under stringent conditions of about 1xSSC, 0.1% SDS, 37°C and has β-ionone ring-2-hydroxylase activity.

2. (Currently Amended) A An isolated gene encoding a peptide selected from the following (a), (b), or (c) or (d):

- (a) a peptide consisting of the amino acid sequence as shown in SEQ ID NO: 4;
- (b) ~~a peptide which consists of the amino acid sequence as shown in SEQ ID NO: 4 having addition, deletion or substitution of one or a plurality of amino acids and has β-ionone ring-2-hydroxylase activity;~~
- (e) (b) a peptide which is isolated from a naturally occurring bacterium and which consists of an amino acid sequence having a 50% or more identity with the amino acid sequence as shown in SEQ ID NO: 4 and has β-ionone ring-2-hydroxylase activity; or

(d) (c) a ~~bacterium derived~~ peptide which is encoded by a DNA consisting of the nucleotide sequence as shown in SEQ ID NO: 3 or a DNA that hybridizes to the full complement of SEQ ID NO:3 hybridizable to a complementary DNA to said DNA under stringent conditions of about 1xSSC, 0.1% SDS, 37°C and has β-ionone ring-2-hydroxylase activity.

3. (Currently Amended) A An isolated microorganism ~~obtainable by introducing comprising~~ the gene according to claim 2 ~~thereinto~~, wherein the microorganism is capable of introducing a hydroxyl group at the position 2 carbon of β-ionone ring.

4. (Currently Amended) A An isolated microorganism ~~obtainable by introducing comprising~~ the gene according to claim 2 and other carotenoid biosynthesis genes ~~thereinto~~, wherein the microorganism is capable of introducing a hydroxyl group at the position 2 carbon of β-ionone ring.

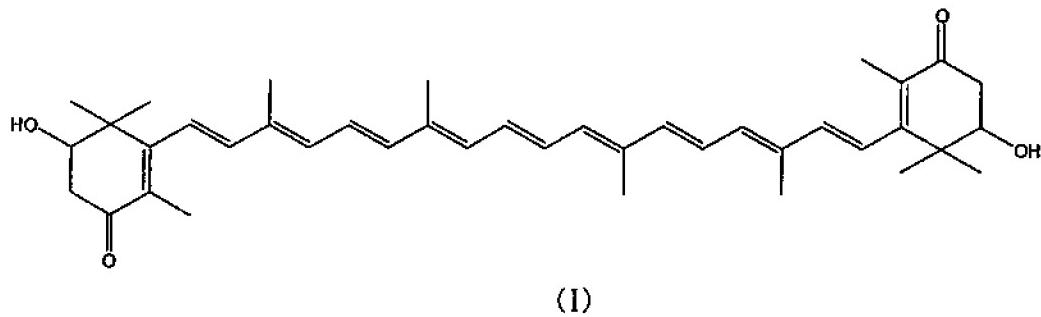
5. (Original) The microorganism according to claim 4, wherein the other carotenoid biosynthesis genes are all or a part of a gene cluster required for synthesizing β-ionone ring-containing carotenoids from farnesyl pyrophosphate.

6. (Previously Presented) The microorganism according to claim 3, wherein the microorganism is *Escherichia coli*.

7. (Previously Presented) A method of preparing a hydroxylated carotenoid, comprising culturing the microorganism according to claim 3 in a medium and obtaining from the resultant culture or cells a carotenoid which is hydroxylated at the position 2 carbon of its β-ionone ring.

8. (Original) The method according to claim 7, wherein the carotenoid which is hydroxylated at the position 2 carbon of its β -ionone ring is β,β -carotene-2-ol (2-hydroxy- β -carotene), β,β -carotene-2,2'-diol (2,2'-dihydroxy- β -carotene), caloxanthin (2-hydroxyzeaxanthin), nostoxanthin (2,2'-dihydroxyzeaxanthin), 2-hydroxy- β,β -carotene-4,4'-dione (2-hydroxycanthaxanthin), 2,2'-dihydroxy- β,β -carotene-4,4'-dione (2,2'-dihydroxycanthaxanthin), 2-hydroxyastaxanthin or 2,3,2',3'-tetrahydroxy- β,β -carotene-4,4'-dione (2,2'-dihydroxyastaxanthin).

9. (Withdrawn) 2,2'-dihydroxy- β,β -carotene-4,4'-dione (2,2'-dihydroxycanthaxanthin) represented by the following chemical formula (I):



(I)

10. (Withdrawn) An antioxidant comprising 2,2'-dihydroxy- β,β -carotene-4,4'-dione (2,2'-dihydroxycanthaxanthin) or 2-hydroxy- β,β -carotene-4,4'-dione (2-hydroxycanthaxanthin) as an active ingredient.

11. (Withdrawn) A gene encoding a peptide selected from the following (e), (f) or (g):

(e) a peptide consisting of the amino acid sequence as shown in SEQ ID NO: 30;

(f) a peptide which consists of the amino acid sequence as shown in SEQ ID NO: 30 having addition, deletion or substitution of one or a plurality of amino acids and has β -ionone ring-3-hydroxylase activity; or

(g) a bacterium-derived peptide which is encoded by a DNA consisting of the nucleotide sequence as shown in SEQ ID NO: 29 or a DNA hybridizable to a complementary DNA to said DNA under stringent conditions and has β -ionone ring-3-hydroxylase activity.

12. (Withdrawn) A microorganism obtainable by introducing the gene according to claim 11 thereinto, wherein the microorganism is capable of introducing a hydroxyl group at the position 3 carbon of β -ionone ring.

13. (Withdrawn) A microorganism obtainable by introducing the gene according to claim 11 and other carotenoid biosynthesis genes thereinto, wherein the microorganism is capable of introducing a hydroxyl group at the position 3 carbon of β -ionone ring.

14. (Withdrawn) The microorganism according to claim 13, wherein the other carotenoid biosynthesis genes are all or a part of a gene cluster required for synthesizing β -ionone ring-containing carotenoids from farnesyl pyrophosphate.

15. (Withdrawn) The microorganism according to claim 12, wherein the microorganism is *Escherichia coli*.

16. (Withdrawn) A method of preparing a hydroxylated carotenoid, comprising culturing the microorganism according to claim 12 in a medium and obtaining from the resultant culture or cells a carotenoid which is hydroxylated at the position 3 carbon of its β -ionone ring.

17. (Withdrawn) A gene encoding a peptide selected from the following (h), (i) or (j):

(h) a peptide consisting of the amino acid sequence as shown in SEQ ID NO: 32;

(i) a peptide which consists of the amino acid sequence as shown in SEQ ID NO: 32 having addition, deletion or substitution of one or a plurality of amino acids and has geranylgeranyl pyrophosphate synthase activity; or

(j) a bacterium-derived peptide which is encoded by a DNA consisting of the nucleotide sequence as shown in SEQ ID NO: 31 or a DNA hybridizable to a complementary DNA to said DNA under stringent conditions and has geranylgeranyl pyrophosphate synthase activity.

18. (New) The isolated microorganism of claim 4, wherein other carotenoid biosynthesis genes is one or more genes selected from the group consisting of *crtE* encoding an enzyme that synthesizes geranylgeranyl pyrophosphate (GGPP) from FPP, *crtB* encoding an enzyme that synthesizes phytoene from two molecules of GGPP, *crtI* encoding an enzyme that synthesizes lycopene from phytoene, *crtY* encoding an enzyme that synthesizes β-carotene from lycopene, and *crtW* encoding β-ionone ring -4-ketolase.